REMARKS

Favorable reconsideration, reexamination, and allowance of the present patent application are respectfully requested in view of the following remarks. The claims have been amended to further clarify the invention. As this amendment does not introduce new issues, but seeks to clarify the claims, applicants earnestly solicit entry thereof.

The amendment of claim 1 to add the term "endogenous" is supported by the specification since it is clear that it is the endogenous RMF gene to *E. coli* which is exemplified in the Examples in the specfication. Therefore, no new matter is added by the foregoing amendments.

The Rejection of Claims 1-6 under 35 U.S.C. §112, 1st Paragraph

The Examiner asserts that claims 1-6 contain subject matter which is not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor had possession of the claimed invention. The Examiner has asserted that the claims are directed toward "Escherichia coli bacterium having any gene from any biological souce having any nucleotide sequence and structure encoding any RMF protein of any amino acid sequence and structure." Applicants have now specifically amended the claims to recite that the rmf gene is endogenous to the *Escherichia coli* bacterium. The endogenous gene from *E. coli* has been reported in the literature and is a known sequence. The Examiner states that the specification does not provide the specfic SEQ ID NO: of the E. coli RMF gene, but only cites to literature references.

Applicants are not required to recite in their specification that which is known in the art. The *E. coli* RMF gene as well as an upstream sequence containing expression control sequences was clearly known in the art at the time of the invention. As exhibit 1, the Yamagishi et al. *EMBO J.* (1993) 12: 625-630, which is cited in the specification and

pointed to by the Examiner, is attached for the Examiner's convenience to demonstrate this fact. Figure 1B of this reference shows the deduced promoter sequence as well sa the sequence of the upstream region.

To determine the promoter region or the transcription factor binding region, one skilled in the art may clearly modify nucleotides in an upstream region and determine the effect on expression, as such procedures are routine in the art. In fact, there are dozens of documents which demonstrate such procedures, including the attached exhibit 2. See Dong et al. *J. Biol. Chem.* (1992) 267:14122-14128. In this reference, the authors report deletion or substitution of four nucleotides in the upstream region, and the effect is analyzed. The results are described in Table IV. In most cases, the expression of the gene was decreased by deletion or substitution of the nucleotides in comparison to the control. The same would clearly be expected for the rmf gene from *E.coli*. Namely, the person of skill in the art can decrease the expression of the gene by modifying an upstream region, the sequence of which is reported in the literature, with the expectation of inactivating the rmf gene.

Applicants also submit that it requires less skill to inactivate a gene as compared to attempting to express a fully active gene. In other words, making changes in a sequence of a gene, or its expression control sequences, requires less experimentation than other types of gene manipulation experiments, since it would be expected that most deletions would disrupt in some way the expression of the gene.

Applicants respectfully submit, therefore, that the claims are fully and adequately described in the specification. The claims encompass a specific method for producing an L-amino acid via bacterial cell culture (all very well-known methods) whereby the gene

encoding the endogenous *E. coli* RMF protein (a known gene and protein sequence) or an expression control sequence thereof (also known) is mutated so that the RMF protein is inactive. See page 13, line 20 to page 17, line 4. The inactivation of the RMF protein by disrupting the RMF gene, or a sequence which controls its expression, and the subsequent ability of a bacterium containing the inactive RMF protein to produce larger quantities of amino acids is the point of novelty. Applicants have discovered that when this known gene is disrupted so that the resulting protein is inactive, L-amino acid production is increased as compared to a bacterial cell having an active RMF protein. This method is clearly described in the specfication, particularly when read in view of the prior art.

The claim is a genus claim, as the Examiner has pointed out, however, but this does not mean that it automatically is not described if only a few species are specifically exemplified. Applicants are not required to describe every species within the genus. The Examiner must also evaluate if there is a common technical feature among the species of the genus which would allow one of ordinary skill in the art to determine that applicants were in possession of the invention as described. The common technical feature of the claimed invention is that disruption of the endogenous *E. coli* rmf gene results in increased production of amino acids by the bacterial cell transformed with the disrupted rmf gene. Claim 1 clearly possesses this common technical feature of the disruption of the known *E. coli* RMF gene. Obviously, disruption of the gene can occur many ways and will result in many variations of the gene. The Examiner is correct in determining that the genus is highly variable, however, again this does not mean the invention is not adequately described. One of ordinary skill in the art would know what actions are necessary to disrupt the gene so that the protein is inactive, since the threshold of expection of success is

very low, as pointed out *supra*. Disruption of the gene and determining other species whereby the gene will express an inactive protein is much easier to do and predict, and within routine experimentation than, for example, determining variants so that the gene will express an active form of the protein.

Furthermore, Applicants were the first to disclose that the production of L-lysine can be improved by disrupting the rmf gene. While the rmf gene is expressed during the stationary phase of the culture and the protein translation activity is decreased in the wild-type strain, it can be inferred that the decrease of protein translation activity is prevented or reduced in a strain in which the normal RMF protein does not function normally (see page 17, lines 15-22). Therefore, clearly the present specification describes that production of an L-amino acid other than L-lysine is also improved in a strain in which the RMF protein does not function normally.

The invention is adequately described, therefore, since all the species do not have to be explicitly set forth in order to sufficiently describe the genus, as long as a common technical feature is present which can be readily ascertained. Limiting the claimed invention to the working examples is not the requirement of the written description, as the Examiner has implied. Applicants must sufficiently describe their invention to ensure the person of skill in the art that they were in possession of their invention. Clearly, applicants have adequately described the invention of disruption of the endogenous *E. coli* rmf gene and the resulting increase in amino acid production and excretion by the *Escherchia coli* cell. This common technical feature binds toghether all of the species of the claimed genus, and hence the invention is adequately described.

The Examiner also states that claims encompasses a genus of methods for making

any L-amino acid, and that the scope includes many L-amino acids with widely physical and chemical structures. Applicants submit herewith a fact-based declaration under 37 C.F.R. §1.132. The declaration presents experimental data showing production of L-glutamic acid by *E. coli* having an inactivated rmf gene. Applicants submit that production of amino acids from bacteria is a very well known technique, and detailed knowledge of the physical and chemical structures of the various amino acids is not necessary to successfully cultivate bacteria and collect a produced L-amino acid. The examples in the specification combined with the example submitted in the attached declaration are clearly sufficient to describe the genus of L-amino acids in the method of the instant claim.

Applicants respectfully request that the rejection be withdrawn in light of the above comments.

Claims 1-6 are also rejected under 35 U.S.C. §112, 1st paragraph for non-enablement. The Examiner asserts that the SEQ ID No. of the inactivated rmf gene is critical or essential to the practice of the invention, but is not included in the claims. Applicants respectfully disagree and set forth the following arguments. The Examiner has stated that the specification provides guidance for transformed *E. coli* host cell containing an inactivated *E.coli* rmf gene which is used in the recombinant production of acid phosphatase and L-lysine. This is exactly what is claimed. Again, it is not evident that the Examiner has recognized that the claims have been limited to the rmf gene of *E. coli*. And particularly now that the claims have been clarified with the addition of the word "endogenous." The Examiner then states that the specification does not provide the SEQ

ID No. of the rmf gene. It is unclear why the Examiner is requiring a SEQ ID No. (or a sequence listing) for a known gene/protein, as this is not required under 37 C.F.R. §§1.821 to 1.825.

There is no requirement to provide a sequence listing for sequences which are known in the prior art. See 37 C.F.R. 1.821(c). It is not necessary to provide information in the specification which is well-known in the prior art, in fact it is preferable to omit such information. See *Spectra Physics, Inc. v. Coherent Inc.*, 827 F.2d 1524 (Fed. Cir. 1987), *cert. denied*, 484 U.S. 954 (1987). The crux of the invention is not the discovery of a new gene or protein sequence, which would require submission of a sequence listing under the rules, but that the disruption of a known gene sequence in an *E. coli* bacterium results in increased production of L-amino acids by the bacterium. The rmf gene from *E.coli* is known in the prior art, as cited in the specification (see page 1, line 20). Therefore, there is no requirement to recite this sequence as part of a sequence listing.

The Examiner has suggested that the claims encompass rmf genes from different biological sources, however, the claims encompass the rmf gene from *E. coli*, which the Examiner has failed to acknowledge. The claims previously presented after first action were limited to the rmf gene from *E. coli*, however, the claims have been amended to further clarify this fact. If the Examiner still believes that the claims are directed to the rmf gene from different biological sources, then applicants respectfully request that the Examiner further explain and clarify his position.

In light of the foregoing arguments, one of ordinary skill in the art would be enabled to practice the steps of the claimed method, and therefore, applicants respectfully submit that the claims are fully enabled and request that the rejection be withdrawn.

Conclusion

For at least the foregoing reasons, Applicant respectfully submits that the present patent application is in condition for allowance. An early indication of the allowability of the present patent application is therefore respectfully solicited.

If Examiner Fronda believes that a telephone conference with the undersigned would expedite passage of the present patent application to issue, he is invited to call on the number below.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the undersigned respectfully requests that she be contacted immediately.

Respectfully submitted,

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